Research

Inactivation of the Anterior Cingulate Cortex Impairs Extinction of Rabbit Jaw Movement Conditioning and Prevents Extinction-Related Inhibition of Hippocampal Activity

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Although past research has highlighted the involvement of limbic structures such as the anterior cingulate cortex (ACC) and hippocampus in learning, few have addressed the nature of their interaction. The current study of rabbit jaw movement conditioning used a combination of reversible lesions and electrophysiology to examine the involvement of the hippocampus and the ACC during acquisition, performance, and extinction. We found that microinfusions of procaine into the ACC did not significantly alter the rate of behavioral learning or the amplitude of hippocampal conditioned unit responses, but that they disrupted the rhythmic periodicity of conditioned jaw movements. During extinction, whereas controls showed a rapid decline in behavioral CRs and active inhibition of hippocampal unit responses, ACC lesioned rabbits showed a persistence of conditioning-related hippocampal activity and behavioral responding. The results show that the ACC can be important for adaptive suppression of conditioned behavior and suggest a crucial physiological modulation of hippocampus by ACC during extinction.

The anterior cingulate cortex (ACC; Brodmann's area 24), a subdivision of the medial prefrontal cortex (mPFC), has been implicated in a wide array of cognitive processes (Devinsky et al. 1995) such as temporal sequencing of behavior (Delatour and Gisquet-Verrier 2001) and reward expectancy (Bush et al. 2002; Shidara and Richmond 2002). Additionally, there are many lines of research suggesting ACC involvement in learning and memory, including the autonomic components of associative learning (Buchanan and Powell 1993), eyeblink conditioning in rabbits (Kronforst-Collins and Disterhoft 1998; Weible et al. 2000, 2003) and in human subjects (Preston et al. 2000), rabbit conditioned jaw movement (CJM) performance (Asaka et al. 2000; McLaughlin and Powell 2001), trace fear conditioning (Han et al. 2003), active avoidance learning (Farr et al. 2000), and discriminative avoidance learning (Gabriel et al. 1991). Extinction, which is believed to be a form of new learning rather than an erasure of an established association (Pavlov 1928), also requires the integrity of areas of the mPFC, including infra- and prelimbic cortices (Milad and Quirk 2002). However, the role of the ACC in extinction of classical conditioning has not yet been thoroughly explored.

Another empirical key to the involvement of the ACC in learning and extinction would be its interaction with other structures known to be crucial for such behaviors, such as the hippocampus. Lesion and recording studies have found that the hippocampus is involved in a variety of learning paradigms, which have been designed to test spatial memory (O'Keefe and Nadel 1978), episodic memory (Eichenbaum et al. 1992), contextual learning (Hirsh 1974), and declarative or explicit memory (Squire 1992). Direct evidence for the involvement of the hippocampal formation in learning has come from studies that combined simple associative learning tasks with simultaneous measurements of hippocampal activity. The hippocampus develops a characteristic acceleration of firing rate in response to nictitating membrane (NM) conditioning. This pattern of hippocampal activity appears early in training, grows over conditioning trials, matches the amplitude-time course of the behavioral response, and precedes this response by several milliseconds (Berger and Thompson 1978). Subsequent investigations have demonstrated that the hippocampus also exhibits this conditioned acceleration of firing rate during the appetitive task of CJM conditioning, with the hippocampal conditioned responses showing a rhythmic pattern that mirrors the behavioral response (Oliver et al. 1993). Importantly, learning deficits in the CJM paradigm are accompanied by attenuated hippocampal responses to the conditioning stimuli (Seager et al. 1997, 1999; Asaka et al. 2000, 2002). Although investigations into hippocampal substrates of associative learning have focused on acquisition rate and performance, there is also evidence for hippocampal involvement in extinction. Specifically, hippocampal lesions interfere with the ability to suppress a response that is no longer adaptive (i.e., no longer paired with a physiologically relevant stimulus). For example, extinction of appetitive Pavlovian conditioning in rats was disrupted by hippocampal ablation (Benoit et al. 1999). Additionally, the results of electrophysiological investigations of hippocampal activity during extinction of the rabbit NM response suggest that the hippocampus is involved in extinction. For example, Berger and Thompson (1982) showed that conditioning-related hippocampal activity decreased in parallel with behavioral extinction of delay NM conditioning. Hippocampal involvement in CJM extinction has not yet been investigated.

Our lab has used the CJM paradigm in several studies to investigate limbic involvement in appetitive associative learning. Unlike the nictitating membrane (NM) classical conditioning paradigm, however, the localization of brain regions that undergo plasticity as the response is learned is not clearly defined. Preliminary evidence has suggested (as it has ruled out for NM conditioning; Krupa et al. 1993) that the locus of the memory

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Article and publication are at http://www.learnmem.org/cgi/doi/10.1101/lm.78404.

11:604–610 ©2004 by Cold Spring Harbor Laboratory Press ISSN 1072-0502/04; www.learnmem.org

trace for the CJM paradigm may reside in the cerebral cortex or in an interconnected network of limbic and cortical structures, which include the anterior cingulate cortex (ACC) and the hippocampus. A consistent finding from previous investigations is that disruption of limbic circuitry slows conditioned response (CR) frequency, leaving unconditioned response (UR) frequency intact. This disruption in CR periodicity has been demonstrated after cholinergic blockade in ACC and medial septum (Asaka et al. 2000) and in aging animals (Seager et al. 1997). These results suggest that the ACC participates in a forebrain circuit involved in making the CR maximally adaptive to the constraints of the learning paradigm.

In summary, previous research suggests that both the hippocampus and the ACC participate in acquisition of conditioned behavior. However, the involvement of the ACC in the extinction of appetitive conditioning and learning-related hippocampal activity has not been thoroughly explored. If the ACC is necessary for extinction, inactivation should lead to the perseverative emission of CRs despite the elimination of the CS–US contingency. Furthermore, if the ACC and hippocampus interact during learning, ACC inactivation might yield informative changes in learning-related activity in the hippocampus. Finally, if the ACC is involved in optimal CJM performance, ACC inactivation should lead to a disruption in CR periodicity, as suggested by previous studies. Therefore, the present study temporarily and selectively suppressed the ACC by administering microinfusions of a local anesthetic directly through chronically implanted cannulae. We then investigated the effects of ACC inactivation on conditioning-related hippocampal unit activity, and behavioral CJM acquisition and extinction. This combination of lesion and recording techniques has been highly successful at establishing neurobehavioral relationships in the rabbit classical conditioning literature and should characterize the contributions of the ACC and hippocampus to conditioning and extinction of JM responses, possibly revealing important details of their interaction.

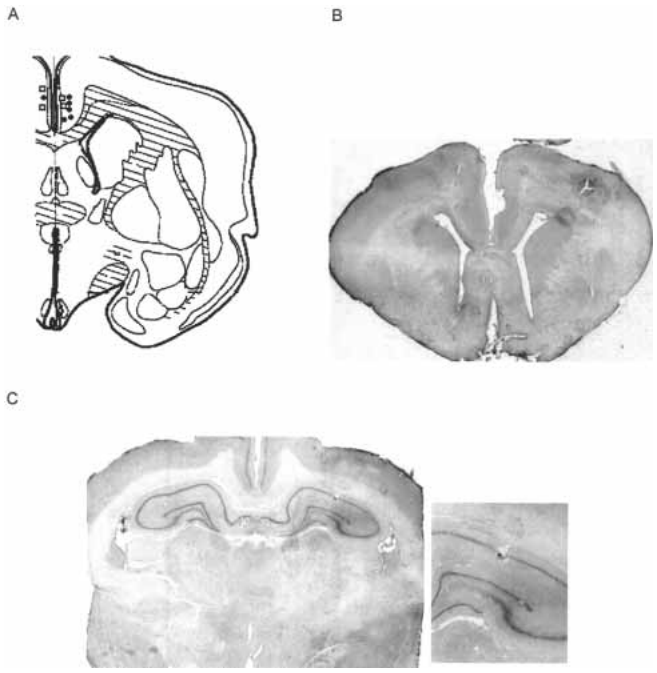

Figure 1 Locations of ACC cannula and hippocampal electrode placements. (*A*) Location of cannulae placements in the procaine (filled circles) and control (open squares) groups. Modified from Girgis and Shih-Chang (1981). (*B*) Nissl-stained section of a representative subject showing the cannula tract in the left ACC (*right* side of picture). (*C*) Nissl-stained section of a representative subject (same as in *B*) showing the electrode location in CA1 of hippocampus. The electrode location appears as a small dark dot in the pyramidal cell layer (Prussian blue reaction; see Materials and Methods). (*Right*) Higher magnification of the electrode site.

RESULTS

Histology

Figure 1, A and B, shows the locations of the cannulae tips in the procaine and control groups. Dye infusions into the cannula before perfusion revealed a 1-mm^3 spread of the infusate into the region surrounding the cannula tip. Therefore, it is likely that our lesions were restricted to the ACC and did not spread to other subdivisions of the mPFC. Figure 1C shows the location of the electrode tip in relation to CA1 from a typical animal. Only recordings from hippocampal electrodes located in CA1 (either stratum pyramidale or oriens) were included in the unit analysis.

Behavior

Our behavioral findings indicate that ACC inactivation led to extinction deficits and, although acquisition rates were comparable to controls, jaw movement frequency was slower in the procaine group than in the control group. This frequency difference was reflected in the selective slowing of the CR part of the JM in contrast to the UR portion. Formal analysis of the jaw movement frequencies revealed that the procaine group exhibited a significantly larger discrepancy between the CR frequency and the UR frequency, $t(7) = 3.0$, $p = 0.02$. This suggests that jaw movements for procaine animals were less adaptive (i.e., did not correspond well to the periodicity of URs and their timing relationship to conditioning stimuli) than those seen in the control animals (see Fig. 2).

Trials to criterion (eight CRs in nine consecutive trials) did not differ significantly between the control (M = 47.0, SD = 27.2) and procaine (M = 62.8, SD = 38.1) groups [$t(6) = 1.19$, $p = 0.28$], demonstrating that inactivation of the ACC did not significantly affect acquisition rate. To assess the rate of extinction, the percentage of CRs per session was compared between the overtraining day and the first day of extinction in the two groups. A 2 × 2 mixed design ANOVA revealed a significant Group by Day interaction, $F_{(1,5)} = 18.01$, $p = 0.008$ (see Fig. 3). Subsequent simple main effects tests revealed that the groups did not differ in percentage of CRs on the overtraining day, $t(5) = 0.76$, $p = 0.48$. However, the control group gave a significantly smaller number of CRs during the first extinction session than the procaine group, $t(5) = 3.55$, $p = 0.02$. Together, these findings suggest that the procaine animals showed normal acquisition rates, but were impaired in extinguishing a previously learned response.

Neural Activity

Histograms depicting unit activity from representative animals (i.e., with standard scores corresponding to the group means) in the procaine and control groups are shown in Figure 4. Both procaine and control animals showed the characteristic increase in hippocampal unit firing rate that accompanies behavioral learning during the acquisition phase (see Berger and Thompson 1978; Oliver et al. 1993; Asaka et al. 2000, 2002). On the first day of extinction, however, an unexpected change in unit activity was observed. Specifically, the hippocampal firing rate was significantly suppressed below baseline in the control animals, which paralleled the inhibition of behavioral responses seen in the session. Conversely, procaine animals, which persisted with high rates of behavioral responding during the extinction session, showed continuing, robust hippocampal conditioned unit responses. These responses, although somewhat attenuated com-

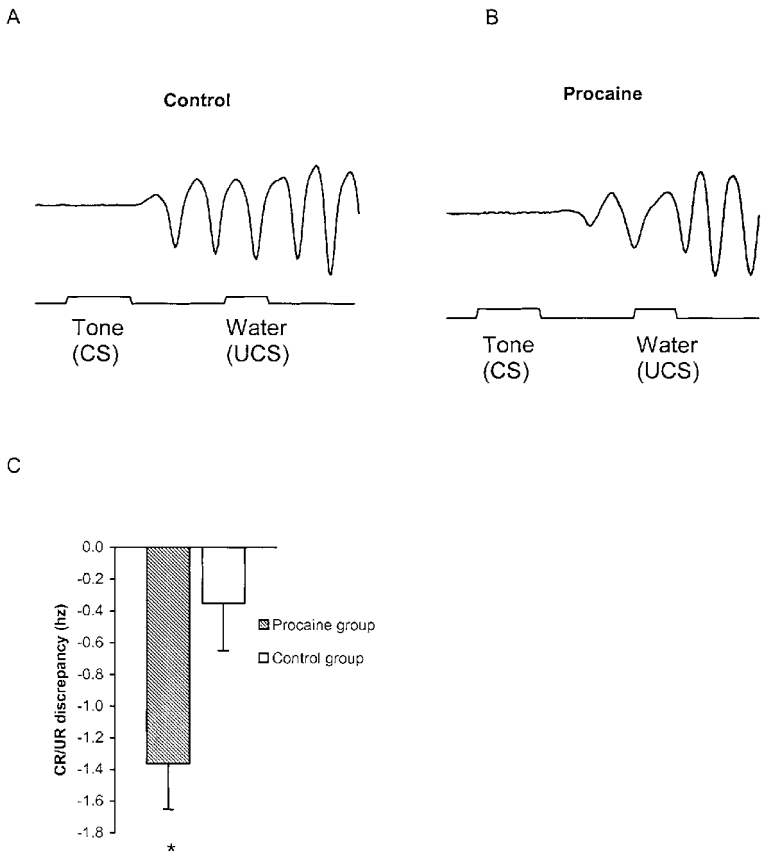

Figure 2 Differences in the frequency of conditioned jaw movements between the procaine and control groups. (*A*) Jaw movement from a representative animal from the control group. (*B*) Jaw movement from a representative animal from the procaine group. (*C*) The group data show that there was a greater CR/UR frequency discrepancy in the procaine group than in the control group. (*) $p = 0.02$.

pared with the overtraining day, still demonstrated the highly reliable and salient increase in firing rate that had accompanied the presentations of the tone during acquisition and criterion performance. The group data are shown in Figure 5. There was a significant Group by Period (see Materials and Method section) interaction, $F_{(4,20)} = 4.95$, $p = 0.006$, with simple main effects tests showing that the unit standard scores were significantly larger in the procaine group during the last half of the tone and throughout the trace period. The pattern of results suggests that ACC inactivation permits continued hippocampal responsiveness to the tone even after the behavioral response is maladaptive because the UCS is no longer predicted by the CS.

DISCUSSION

The results of the current investigation indicate that CJM extinction is accompanied by an immediate and active suppression of hippocampal conditioned unit responses. ACC inactivation prevents this extinction-related suppression and, correspondingly, leads to behavioral extinction deficits. Acquisition rate and the learning-related acceleration of hippocampal unit activity were unaffected by the reversible lesion. However, not surprisingly given the results from previous manipulations of forebrain systems, procaine animals displayed disruptions in the periodicity of conditioned (but not unconditioned) jaw movements.

The fact that ACC inactivation interferes with the ability to extinguish a learned response is consistent with other investigations suggesting a role of the prefrontal cortex (which includes ACC and other subregions) in response suppression. Most of these experiments used fear conditioning, and interpreted prefrontal activation during extinction as a "safety signal" (Milad and Quirk 2002). The current results instead suggest a more general role of at least one area of the prefrontal cortex (the ACC) in response suppression even when the motivational context of the task is reward rather than fear. An important caveat is that our results may reflect the effects of temporarily inactivated fibers of passage through the ACC (e.g., the cingulum bundle). Therefore, although we are confident that the infusate did not spread to other regions of the mPFC, we cannot rule out the possibility that regions such as the parahippocampal region, presubiculum, retrosplenial cortex, and premotor areas, which are targets of cingulum bundle fibers (Mufson and Pandya 1984; see also Aggleton et al. 1995) were partially deafferented by our infusion procedure. Even in that case, our data are among the first to demonstrate the modulatory effect of this system on appetitive classical conditioning. Future studies could use a more selective reversible lesion method, perhaps GABA receptor activation, to suppress only ACC activity without disrupting fibers of passage.

In parallel with the behavioral results and consistent with our past studies (see Asaka et al. 2000), procaine administration to the ACC did not significantly disrupt conditioning-related hippocampal activity during the acquisition phase of the experiment. However, during the extinction phase of the current study, a profound decrease in conditioned hippocampal unit activity was seen in control animals. In fact, most animals showed a tone-evoked suppression of hippocampal activity below baseline levels on extinc-

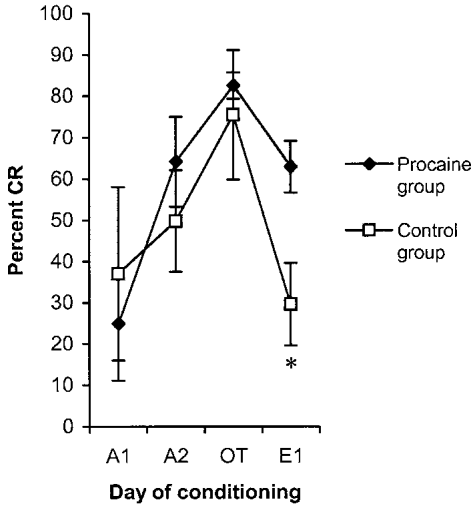

Figure 3 Percentage of CRs given across conditioning sessions. The procaine and control animals have similar percentages of CRs across acquisition days 1 and 2 (A1 and A2) and on the overtraining day (OT). However, on extinction day 1 (E1), the procaine group gave a significantly higher percentage of CRs than the controls. (*) $p = 0.02$.

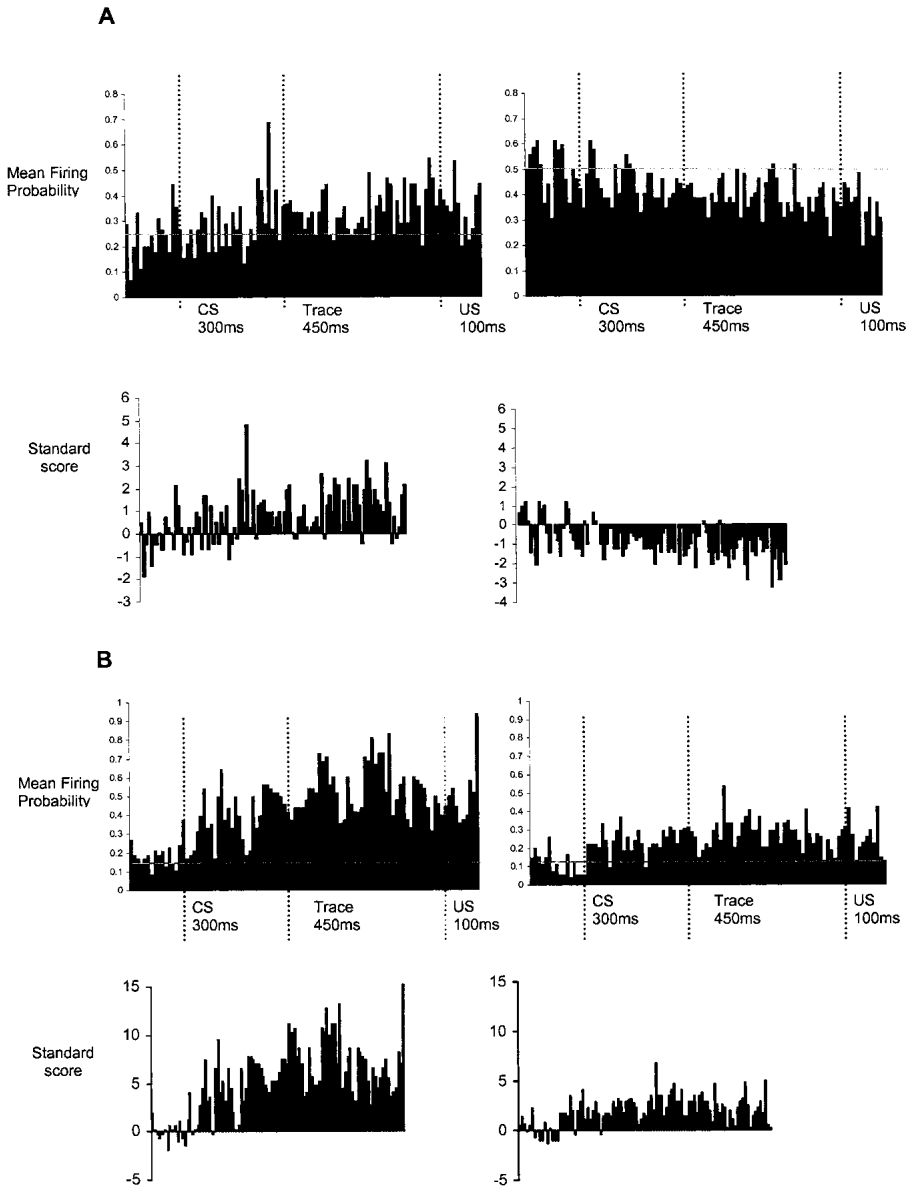

Figure 4 Examples of unit standard score histograms (*bottom*) and peristimulus time histograms (*top*) from representative animals from the control (*A*) and procaine (*B*) groups on overtraining day (*left*) and the first day of extinction (*right*). Notice the suppression of hippocampal unit responses on extinction day in the control animal, especially during the trace period, which is absent after ACC inactivation. Vertical dashed lines denote tone onset, offset, and water onset. Solid horizontal lines denote pretrial baseline firing rates.

tion day 1, suggesting that the hippocampus was being actively inhibited when the animal was learning to override the behavioral response because the tone was no longer followed by water delivery. Although ACC inactivation did not completely prevent quantitative changes in hippocampal conditioned unit activity from the overtraining to extinction day, standard scores did not decrease as dramatically in procaine animals as in controls. In terms of qualitative effects on hippocampal responses, the procaine and control groups differed markedly in the occurrence or absence of conditioned unit activity. Hippocampal responses persisted robustly in the procaine group, with only one animal having slightly negative standard scores confined to the last two trace periods. In contrast, the active inhibition in the control group was seen in all subjects during at least some periods of the trial, with most excitatory responses completely suppressed. We did not observe any difference between hippocampal recordings ipsilateral and contralateral to the infusion site; however, this lack of a difference may be a result of the small lesion size. It is likely that larger lesions would have shown a differential suppressive effect on the ipsilateral and contralateral hippocampus (see Dolleman-Van der Weel et al. 1997). Although more research is required to characterize the nature of interaction between the ACC and hippocampus, our group difference in hippocampal suppression suggests that an active inhibition of hippocampal conditioned responding to the tone CS is mediated in part by the ACC.

Our results are consistent with anatomical studies that have documented indirect but clear pathways between the ACC and hippocampus. Although several studies have revealed direct connections between the prefrontal cortex and hippocampus, these connections appear to be largely unidirectional from hippocampal regions CA1 and subiculum and restricted to areas of the ventral mPFC, specifically infralimbic and prelimbic cortex (Ferino et al. 1987; Carr and Sesack 1996). The more dorsal areas of the mPFC such as ACC have a more indirect anatomical relationship to the hippocampus via parahippocampal regions, receiving projections from the dorsolateral entorhinal area (Van Eden et al 1992; Delatour and Witter 2002) and, in turn, projecting to the perirhinal cortex (Sesack et al. 1989). In addition, a recent study suggests that the nucleus reuniens (RE) may be a key relay point between the mPFC and hippocampus (Vertes 2002). Anatomical tracing techniques revealed that RE, which is known to be the major source of thalamic projections to the hippocampal formation (Dolleman-Van der Weel et al. 1997), is strongly innervated by all subdivisions of the mPFC, including the ACC (Vertes 2002). If, during extinction, prefrontal regions exert their inhibitory influence on hippocampus through a relay in RE, one would expect to see evidence of an excitatory connection on hippocampal interneurons from RE. Indeed, RE projections to CA1 form excitatory connections on inhibitory interneurons (Wouterlood et al. 1990), suggesting that excitatory input from RE can exert a strong inhibitory effect on CA1 pyramidal cells through activation of inhibitory interneurons. Therefore, there is anatomical support for our observation that suppression of ACC with procaine could, through indirect connections via either RE, parahippocampal regions or both, deprive the hippocampus of input needed for optimal performance and extinction of CJM.

Several investigations have reported ACC activation during acquisition of associative learning. A recent recording study

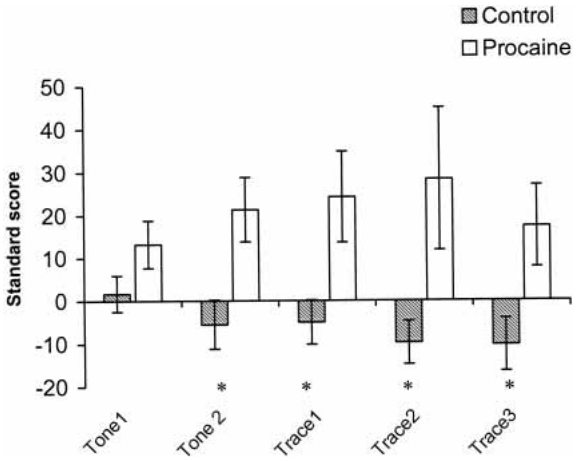

Figure 5 Unit standard scores for the procaine and control groups over the five periods of the trial. The procaine group maintained a significantly higher level of hippocampal conditioned unit activity than the control group. (*) $p < 0.05$.

showed that the ACC exhibits conditioning-related acceleration of firing during NM conditioning similar to that seen in rabbit hippocampus (Weible et al. 2003). Similarly, single unit activity in prefrontal cortex (including ACC) showed heterogeneous, but robust responses to conditioning stimuli during discriminative jaw movement conditioning (McLaughlin et al. 2002). Paralleling these results, event-related activity has been reported in the ACC of human subjects during eyeblink conditioning during CR performance, suggesting that the ACC is activated in human subjects during the formation of an association between the CS and the US (Preston et al. 2000). Although we did not observe differences in conditioned unit activity between the procaine and control group during acquisition, other studies have suggested that the ACC is critically involved in acquisition of associative conditioning. Acquisition deficits in eyeblink conditioning were observed following relatively larger bilateral ACC lesions (Kronforst-Collins and Disterhoft 1998). It is likely that the inconsistency between the two studies is due in large part to the more extensive lesions in the previous study. Therefore, the fact that we observed only extinction deficits after a relatively small unilateral lesion suggests a more crucial involvement of the ACC in extinction than in acquisition. Together, prior studies suggest that the ACC, like the hippocampus, is activated during acquisition, but our results suggest that the ACC may become more crucial in the extinction phase of the experiment. It is unclear whether the active suppression seen in hippocampus during extinction originates in the ACC or in the relay regions like the RE. The most straightforward interpretation of observations of both ACC and hippocampal unit responses during acquisition is that activity in these areas is not antagonistic or mutually exclusive. If this is not the case, there remain open questions about a selective role for the ACC in jaw movement extinction or about possible intermediate structures that convert ACC responses into an inhibitory effect on hippocampus when extinction training begins. The mechanism of inhibition within the hippocampus is presumably activation of inhibitory interneurons from hippocampal afferents such as RE, which leads to the suppression of CA1 pyramidal cell activity. Future studies could investigate the nature of the extinction-related suppression of hippocampal activity by examining the relationship between mPFC, RE, and hippocampus during acquisition and extinction using lesions, recordings, or a combination of both.

The finding that CRs in the procaine group were significantly slower than the control group is consistent with the results of a previous study showing the same effect on jaw movement frequency following cholinergic blockade in the ACC (Asaka et al. 2000). Moreover, demonstrations of disruptions in CJM frequency have been shown after systemic administration of a cholinergic blocker (Seager et al. 1999), and in aged animals (Seager et al. 1997). According to Zeigler (1989), motivational state has a significant impact on the frequency of the ingestive pecking response in pigeons, with higher frequencies corresponding to higher levels of motivation. To the extent that this may be true for rabbit jaw movement conditioning, the slower CR frequencies in the procaine group may reflect a loss of the ability of the ACC to encode the motivational relevance of the conditioning stimuli and project this information to the hippocampus. Additionally, the ACC may provide modulatory input to jaw movement control systems themselves through its projections to premotor areas (Mufson and Pandya 1984). Consistent with the idea of this modulation, electrical stimulation of the ACC in the anesthetized rabbit yields fictive movements restricted to the jaw and lip (Griffin et al. 2002).

The goal of the current investigation was to characterize both the involvement of the ACC in trace-conditioned jaw movement and the degree of interaction between the ACC and hippocampus during conditioning and extinction of this hippocampus-dependent appetitive task. Understanding the dynamic interactions between the hippocampus and the cortex, especially the prefrontal cortex, may provide valuable insight into not only the functional roles of limbic structures, but also the distributed nature of learning and memory processes throughout the forebrain.

MATERIALS AND METHODS

Subjects

Subjects were 10 New Zealand White rabbits (*Oryctolagus cuniculus*) supplied by Myrtle's Rabbitry (Thompson Station, TN). All animals were maintained on a 12:12 light–dark cycle, with training conducted during the light phase. Animals were allowed free access to food in their home cages. A water regulation schedule, in which animals were given 2 h per day of access to water in their home cage, began 3 d prior to the first conditioning session and continued until the final conditioning session. During water restriction, a metal cap was placed over the water sipper, preventing the animals from drinking. Body weights were monitored throughout water restriction to ensure that no dramatic (>10%) loss of weight occurred. All procedures involving animals were approved by the Miami University Institutional Animal Care and Use Committee. Of the 11 animals (seven in the procaine group and four in the control group) included in the study, one animal from the procaine group was excluded because the cannula was located in the longitudinal fissure. This left six animals in the procaine group and four animals in the control group. One procaine animal was excluded from the JM frequency analysis because of inadequate water deprivation prior to the first conditioning session, leaving five animals in the procaine group and four animals in the control group. One control animal was excluded from the remaining behavioral analyses because of mechanical problems with transducing the jaw movements that occurred prior to the animal reaching the 8/9 behavioral criterion, leaving five animals in the procaine group and three animals in the control group. After histological verification of accurate cannula and electrode placements and the criterion of a signal/noise ratio of at least 3:1 in our hippocampal recordings, we were left with four hippocampal recordings in the control group and three recordings in the procaine group.

Electrode and Cannula Implantation

All rabbits were anesthetized with ketamine (50 mg/kg i.m.) and xylazine (10 mg/kg i.m.) and implanted with bilateral hippocampal electrodes (size 00 stainless steel insect pins coated with epoxylite [Epoxylite Corp.] except for 50–70 microns at the tip). Prior to electrode implantation, a small hole was made in the left cheek using a leather punch and a nylon tube was inserted for delivery of the water US during conditioning. The electrodes were positioned according to stereotaxic coordinates (Girgis and Shih-Chang 1981; 4.5 mm posterior to bregma, 5.5 mm lateral to the midline suture, and ~3.0 mm ventral to dura) and by monitoring activity from the electrode tip during implantation. A single guide cannula (Plastics One Inc.) was implanted in either the left or right ACC according to stereotaxic coordinates (Girgis and Shih-Chang 1981; 1.5 mm anterior to bregma, 0.5 lateral to the midline suture, and 4.0 mm ventral to dura). After implantation, the electrode wires were soldered into a 9-pin amphenol connector, the electrodes and cannula were cemented into place with dental acrylic, the incision was sutured, and the animal was transported to the recovery area.

Training

After 5 d of postsurgical recovery, 3 d of water regulation, and two 30–45-min sessions of adaptation to the restraint apparatus and conditioning chamber, animals in both the procaine and control groups began trace CJM conditioning. The paradigm consisted of a 300-msec, 1-kHz, 80-dB tone followed by a 450-msec trace interval and a 1-cc 200-msec intraoral delivery of water. Each session lasted ~90 min and included six blocks of eight paired trials (48 total), paired trials, and 1 tone-alone trial (nine total). The intertrial interval was 60 sec. Rabbits were trained until they reached a behavioral criterion of eight conditioned responses (CRs) out of nine consecutive trials, which is conventionally thought to be the point of asymptotic responding (Gormezano et al. 1987). A CR is defined as at least 0.5 mm of movement of the jaw occurring after tone onset and before water onset on paired trials and occurring after tone onset on test trials. Jaw movements were transduced by attaching a potentiometer arm to the jaw with masking tape. Calibration was done before each training session to determine the voltage change in the potentiometer output that corresponded to 0.5 mm of movement. The animals were each given one additional day of conditioning (overtraining). Following the overtraining day, the animals were given 3 d of extinction training, in which the tone CS was no longer followed by the water US. Extinction was measured by the percentage of CRs given during each 54-trial session.

Animals were trained in an electrically shielded sound-attenuating chamber. Behavioral and neural activity was amplified using customized bioamplifiers and transduction system and recorded on VCR tape (A.R. Vetter, Model 420) for off-line analysis. Stimulus presentation was controlled by a customized software program (Labview, National Instruments Corporation) and custom interface system (programmed by Lynn D. Johnson, Miami University). Synchronization pulses from the computer (which mark stimulus onset and offset) were also recorded on tape for use in offline analysis.

Infusion Procedure

The cannula system consists of a guide cannula, which is implanted such that the tip is situated 1 mm above the target infusion site and is fixed permanently to the skull with dental acrylic. A dummy cannula is inserted into the guide cannula and left in place until the time of the infusion to prevent clogging. The injection cannula is placed into the guide cannula for the infusion and projects 1 mm out the bottom of the guide cannula, directly into the target area. Immediately prior to conditioning, the dummy cannula was removed and an injection cannula was placed into the guide cannula for delivery of 1 µL of procaine (20% by volume in saline) for the procaine animals ($N = 7$) or saline for the control group ($N = 4$). The injection cannula was connected with PE50 tubing to a 10-µL Hamilton syringe, which was placed into an infusion pump (KD Scientific Model 100) that delivered the infusate over a 2-min period. After the infusion, the injection cannula was left in place for 1 min and then removed. The dummy cannula was replaced into the guide cannula, and training began ~5 min later.

Histology

At the end of the experiment, animals were lightly anesthetized, and a small marking lesion was made by passing a 200-µA, 10-sec DC current through each recording electrode (Grass Stimulator Model SD-9; Grass Instruments). Then 1 µL of Fast green (Sigma) dye was infused into the cannula to determine spread of the drug. Animals were then given an overdose of sodium pentobarbital (Euthasol, 0.2205 mg/kg, i.v.) and perfused intracardially with saline (0.9%) and formalin (10%) solutions. The brains were removed, sectioned with a cryostat, embedded on gelatin-coated slides, stained with Prussian blue to mark the locations of the electrode tips, and counterstained with Safranin (Sigma). Slides were examined using a compound microscope (Nikon) for verification of cannula and electrode locations. Photographs of histological sections were taken using a Nikon Super Coolscan scanner. Only animals with electrodes in CA1 (stratum oriens or stratum pyramidale) and with the cannula tip in the ACC were included in the study.

Data Analysis

The difference in acquisition rate (number of trials to reach the 8/9 CR criterion) between the procaine and control group was analyzed using an independent groups *t*-test. The differences between groups in percent CRs given during acquisition and extinction sessions were assessed using a 2×6 mixed ANOVA with group as the between-subjects factor and day as the within-subjects factor. Transduced behavioral responses from the potentiometer were filtered and converted to digital values by Labview software. Six CRs and six URs from the first day of training were chosen at random for each rabbit for the jaw movement frequency analysis. Autocorrelations were conducted to determine the frequency of the CR and UR components of the selected responses. The frequencies of the six responses of each type were then averaged together to give a characteristic jaw movement frequency for each animal. These procedures for jaw movement frequency analysis have been used previously in our lab (see Asaka et al. 2000). For each animal, a discrepancy score (UR frequency − CR frequency) was calculated, and differences between the procaine and control groups were assessed using an independent groups *t*-test. The discrepancy score reflects the degree of similarity between the CR and the UR on each trial. CRs with the closest resemblance to URs were presumed to be the most adaptive.

Multiple-unit activity from the electrodes was band-pass filtered (500–5000 Hz, Krohn-Hite Model 3700 filter; Krohn-Hite Corp.) and passed through a window discriminator, which separates the largest spikes in the unit activity from background activity (signal-to-noise ratio of 3 to 1 or greater). A computer sampled at the rate of 12 kHz and computed the number of spikes crossing the window discriminator threshold beginning at the initiation of each trial and ending 1 sec later. Each trial was divided into 100 10-msec bins, and the number of threshold crossings were counted for each bin. To obtain a session average, the bins were averaged across all trials for each training session, giving us peristimulus time histograms, with the height of each bin indicating the probability of a threshold crossing for each 10-msec portion of the trial averaged across the training session. Hippocampal neural activity was quantified, computing standard scores from each animal's daily histogram by subtracting the average bin height of the pre-CS period from the height of each of the 100 bins and dividing by the standard deviation of the pre-CS period. Positive standard scores indicate an acceleration of firing rate over baseline levels, and negative standard scores indicate a suppression of firing below baseline levels. The tone and trace portions of the histogram were divided into five equal 150-msec periods, with each period summarizing neural responses during a portion of the training trial as follows: Periods

1 and 2, first and last halves of tone, respectively; Periods 3, 4, and 5, first, second and third trimesters of trace, respectively. A total score was calculated for each period for each animal by adding together the individual standard score values for the appropriate 15 bins. To test differences between groups, a 2×5 mixed design ANOVA was used in which group was a between-subjects factor and period was a within-subjects factor.

ACKNOWLEDGMENTS

The authors thank Phillip Best, Alan Pantle, Michael Nagy, and Phyllis Callahan for helpful suggestions on an earlier version of this manuscript. We also thank Lori Isaacson, Ryan Darling, Brad Haverkos, and Rachel Vesco for providing technical assistance.

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Received April 2, 2004; accepted in revised form July 13, 2004.